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A novel immunoassay using platinum nanoparticles, silver enhancement and a flatbed scanner

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Abstract

This study reports a potentially rapid and convenient immunoassay using antibody-platinum nanoparticle (Ab-PtNPs) conjugates as a reporter molecule and a flatbed scanner for detecting immuno-reaction and measuring of the immuno-reaction signal. This study was based on the sandwich immunoassay (three-layer format) which contained a primary antibody, test antigens and a secondary antibody. The results showed that the silver precipitation phenomenon was catalyzed by Ab-PtNPs conjugates. The changing color of reaction could easily be observed by naked eye or scanner. The silver enhancement reaction, a signal amplification method in which silver ions are reduced to silver metal, is introduced to magnify the detection signal. The relationship between sample concentration and detection signal was discussed. And the detection limit (sandwich assay) for the sample antigen was 10^{-1} ng/mL. Using a flatbed scanner, Ab-PtNPs conjugates and a silver enhancement reaction, a new immunoassay is constructed.

Keywords: Immunoassay, platinum nanoparticles, Immuno-reaction, falted scanner.

1. Introduction

Immunoassay based on a specific interaction between an antibody and a complementary antigen is a powerful analytical method for clinical diagnosis, and environmental monitoring. Based on the materials of protein labeling, it can be classified as radioimmunoassay [1], fluorescent dyes immunoassay [2], chemiluminescence's immunoassay [3], enzyme-linked immunosorbent assay (ELISA), and so on [4, 5].

Recently, metallic nanoparticles (e.g. gold and silver colloids) have been successfully used to the label technology because of their easily controlled size, and high biocompatibility with antibodies, proteins, RNA and DNA [6]. The immunogold silver staining (IGSS) technique has been applied to localize antigens in cells or tissues, and recently the method was found to have the capability to increase the assay sensitivity of DNA array [7, 8]. To date no effort has been made to apply IGSS technique coupled with 4 nm PtNPs to improve the performance and sensitivity of immunoassays. Therefore, we employed a flatbed scanner, Ab-PtNPs conjugates and an IGSS technique to construct a novel immunoassay.

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2. Material and method

2.1 Principle

The schematic principle of the developed method is illustrated in Fig. 1. The whole framework of the study is based on sandwich-type immunoassay (three-layer format) which is designed for qualitative and quantitative analysis. For sandwich immunoassay, first antibodies are bound on the glass slides then test antigens are bound to the first antibodies. Excess unbound antigens are removed by the washing solution (PBST (Phosphate-buffered saline + 0.05% Tween 20)). Upon the addition of the IgG-PtNPs conjugates to the chip, they will bind to the antigens which are already bound to the first antibodies, resulting in formation of a sandwich-type complex. In this way, we could analyze the relationship between concentrations of test antigen and variation of the grayscale to achieve a qualitative analysis and get a precisely limitation of this novel proposed model.

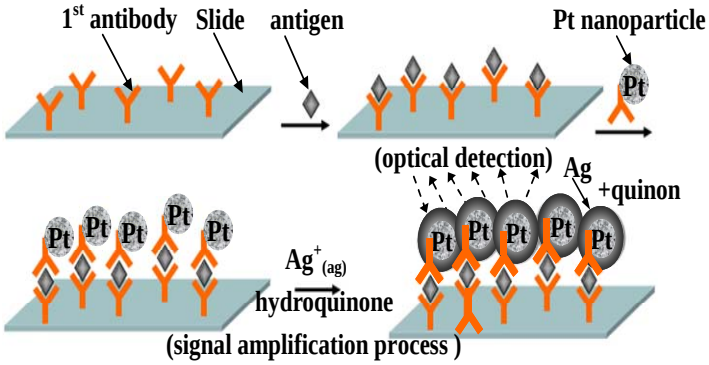

Fig. 1. Schematic drawings of the signal amplification method for immunoassay. Silver ions are reduced by hydroquinone to bulk silver metal, which leads to the silver covering on the platinum nanoparticle surfaces.

2.2 Methods

IgGs (1st Ab) were coated on the slide as the first layer for binding sample antigens protein A. Then protein A of various concentrations was immobilized as the second layer by the IgG coating layer. The strong affinity between IgGs and protein A could offer more immobilization for sample protein A of lower concentrations. Hence, the concentrations of the sample antigen (protein A) in the sandwich format were 10^4, 10^3, 10^2, 10, 1, 10^{-1} and 10^{-2} ng/mL. Each experiment was performed three times to ensure the accuracy of the results. Finally, the corresponding IgG-PtNPs conjugates were added responsible for producing the optical detection signal

The system is shown in Figure 2: (a) reaction well using PDMS as the material on glass slide was patterned with a circular area, (b) the scanner (Scanjet 3400C) was used to scan and capture the reaction signal, (c) a personal computer with Adobe Photoshop installed was used to analyze the gray level data, and (d) the analyze result by sigmaplot software.

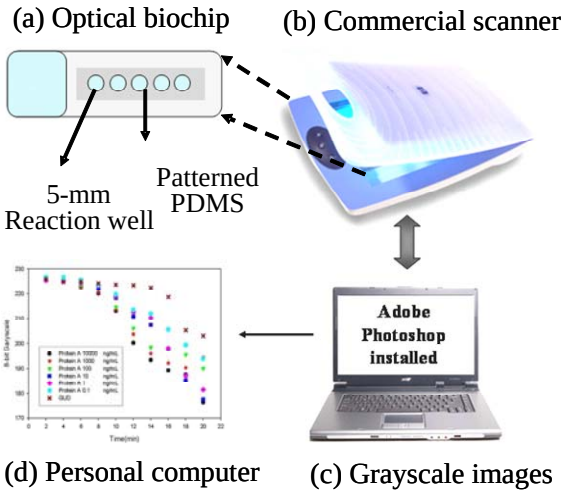

Fig. 2. The whole detection system including (a) the PDMS wells are on glass slide for reaction region, (b) the flatbed scanner is used to get experimental gray scale value, (c) the Adobe Photoshop program is used to analyze and acquire the gray level data, and (d) the analyze result by sigmaplot software.

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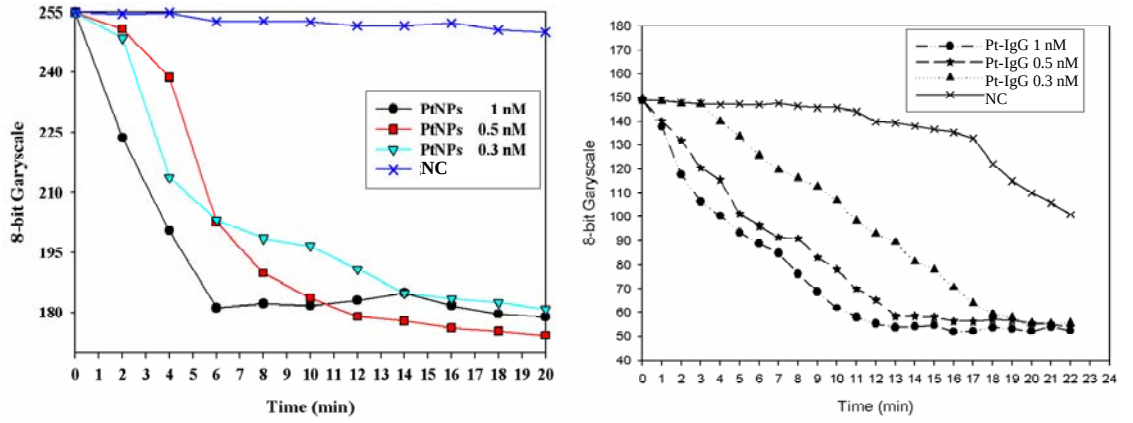
3. Results and discussions

In order to test the applicability of the present assay format, PtNPs were employed as a model. Results show that the PtNPs reduced silver ion to silver metal in the presence of a silver enhancer solution. Figure 3(a) shows the real-time color change of the different concentration PtNPs in the presence of the silver enhancer solution. The PtNPs color change could be observed with the naked eyes within a period from 2 to 14 minutes. For the highest concentration of PtNPs solution, the gray level values reached the saturation point promptly after about 6 minutes, indicating that all silver ions appeared to be reduced to the silver metal by catalysis. When the concentrations were lowered to 1.0 nM, the change profiles of the gray level values were similar to each other, indicating that the saturation of PtNPs concentration had been reached.

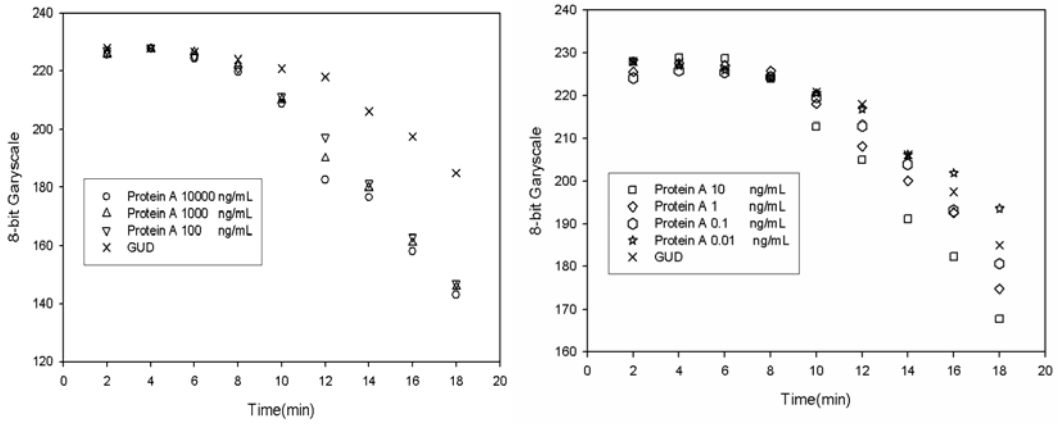
The applicability of the present assay format, IgG-PtNPs conjugates were employed as a model. Results show that the IgG-PtNPs conjugates reduced silver ion to silver metal in the presence of silver enhance solution. It is evident that IgG-PtNPs conjugates retain the ability to reduce silver ions. Figure 3(b) shows the real-time color change of the different concentration IgG-PtNPs in the presence of the silver enhancer solution. The color change could be observed with the naked eyes within a period from 2 to 14 minutes at an IgG-PtNPs concentration of 1.0 nM. For the highest concentration of IgG-AuNPs solution, the gray level values reached the saturation point promptly after about 12 minutes, indicating that all silver ions were reduced to the silver metal by catalysis. When the concentrations were lowered to 1.0 nM, the change profiles of the gray level values were similar to each other, indicating that the silver precipitation ability of IgG-PtNPs concentration had been reached and saturated.



(a) (b)

Fig. 3. Different concentrations of PtNPs and PtNPs-IgG in silver enhancement reaction have different 8-bit grayscale variation. The 1 nm PtNPs has reached the saturation point at 6mins. The highest PtNPs-IgG concentration has the saturation point at 12mins.

In sandwich immunoassay experiments, the concentration of first antibody was fixed 100 μg/mL, and the concentration of antigen was set from 10^{4} to 10^{-2} ng/mL. The antigen concentration was respectively mapped to each detected gray level value (from 10^{4} ng/mL to 10^{-2} ng/mL) as shown in Fig. 4, which provides the relation between the sample concentrations and the detection signals. The gray level value of 10^{-2} ng/mL concentration approaches the gray value of the control experiments (control: 204.55, 10^{-2} ng/mL: 202.98), indicating that the detection limit for sandwich format is 10^{-1} ng/mL.



(a) (b)

Fig. 4. The dynamic ranges of different concentrations could be determined from gray-scale value. According to the results, the detection limit for sandwich format is 0.1 ng/mL at 12 minutes.

4. Conclusions

We have successfully developed a novel immunoassay method which gains a lot of advantages. Labeling antibody targets with PtNPs rather than enzyme, fluorescence, or immunogold substantially provided an interesting and attracting method which has the potential to improve the conventional systems. In this study we proposed an immunoassay based on a flatbed optical scanner to measure the immuno-reaction signal, with PtNPs as a label of antigen or antibody and as a catalyst for silver precipitation, and with the silver enhancement reaction to magnify the detection signal. The detection limit for sample antigen is 10^{-1} ng/mL and the detection reaction time is about 12 mins. This method has several advantages: (1) the implementation of an assay is easy, (2) only a small amount of reagent is required (as little as 28 μL of sample protein per well detection), (3) it is faster than AuNPs and

AgNPs (optical detection within 12 to 20 min), and (4) convenient and low-cost detection (an ordinary desktop scanner). This method has many potential usages for protein microarray research and clinical diagnosis.

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